

| | L # | Hits | Search Text | DBs | Time Stamp |
|---|-----|------|-----------------------------|--------------------|---------------------|
| 1 | L1 | 2 | luciferin near4 regenerat\$ | USPAT; US-PGPUB | 2003/08/06 14:21 |

US-PAT-NO: 5891659

DOCUMENT-IDENTIFIER: US 5891659 A

TITLE: Bioluminescent adenosine phosphate ester assay and reagent

DATE-ISSUED: April 6, 1999

INVENTOR-INFORMATION.

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|---------------------|-------|-------|----------|---------|
| Murakami, Seiji | Noda | N/A | N/A | JP |
| Sakakibara; Tatsuya | Noda | N/A | N/A | JP |
| Eisaki; Naoki | Noda | N/A | N/A | JP |
| Nakajima; Mctoo | Noda | N/A | N/A | JP |
| Imai; Kazuhiko | Tokyo | N/A | N/A | JP |

APPL-NO 08/ 805613

DATE FILED: February 26, 1997

FOREIGN-APPL-PRIORITY-DATA:

| COUNTRY | APPL-NO | APPL-DATE |
|---------|----------|---------------|
| JP | 8-070911 | March 4, 1996 |

US-CL-CURRENT 435/8, 435/15, 435/21

ABSTRACT:

There is provided a bioluminescence reagent comprising at least pyruvate orthophosphate dikinase, phosphoenolpyruvic acid, pyrophosphoric acid, magnesium ion or another metallic ions, luciferin and luciferase, which reagent is such that the amount of luminescence is maintained in a high level and moreover stably without decaying for a long time in a bioluminescence reaction, and there is provided a method for quantitatively determining an adenosine phosphate ester or a substance taking part in the ATP conversion reaction in high sensitivity and high accuracy using an inexpensive and simple measuring apparatus.

7 Claims, 5 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 5

----- W.C -----

Brief Summary Text - BSTX (25):

The present inventors have intensely made sequential researches to solve these problems, and they have found that when a reagent comprising ATP regenerating enzyme, substrates of ATP regenerating enzyme, magnesium ion, luciferin and luciferase is reacted with a sample containing an adenosine phosphate ester, the amount of luminescence is maintained in a high level and moreover stable without decaying for a long time, and it gets possible to quantitatively determine the adenosine phosphate ester in high sensitivity and high accuracy using an inexpensive and simple measuring apparatus wherein said ATP regenerating enzyme catalyzes the formation of ATP from AMP.

US-PAT-NO: 5814504

DOCUMENT-IDENTIFIER: US 5814504 A

TITLE: Protein involved in regenerating firefly luciferin

DATE-ISSUED: September 29, 1998

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|-----------------|-------|-------|----------|---------|
| Kajiyama, Naoki | Chiba | N/A | N/A | JP |

APPL-NO: 08/ 869996

DATE FILED: June 5, 1997

PARENT-CASE:

PROTEIN INVOLVED IN REGENERATING FIREFLY LUCIFERIN

This application is a continuation of Provisional application No. 60/024,771, filed Aug. 22, 1996.

US-CL-CURRENT: 435/189, 435/8, 530/417

ABSTRACT:

A purified protein having a molecular weight of 40 kD by SDS-PAGE that produces firefly luciferin when combined with D-cysteine and firefly oxy luciferin and isolated from firefly species is provided, as well as methods of making and using the protein for the continuous regeneration of firefly luciferin.

20 Claims, 0 Drawing figures

Exemplary Claim Number: 1

Abstract Text - ABTK (1):

A purified protein having a molecular weight of 40 kD by SDS-PAGE that produces firefly luciferin when combined with D-cysteine and firefly oxy luciferin and isolated from firefly species is provided, as well as methods of making and using the protein for the continuous regeneration of firefly luciferin.

TITLE: Protein involved in regenerating firefly luciferin

Protein involved in regenerating firefly luciferin

Parent Case Text - PCTX (1):

PROTEIN INVOLVED IN REGENERATING FIREFLY LUCIFERIN

Brief Summary Text - BSTX (1):

PROTEIN INVOLVED IN REGENERATING FIREFLY LUCIFERIN

Brief Summary Text - BSTX (4):

The present invention relates to a protein involved in regenerating luciferin.

Brief Summary Text - BSTX (8):

Under existing circumstances, no protein acting on oxyluciferin to regenerate luciferin as the luminescence substrate has been isolated and purified.

Brief Summary Text - BSTX (11):

The object of the present invention is to provide a protein having the ability to regenerate luciferin by acting on oxyluciferin and D-cysteine.

Brief Summary Text - BSTX (12):

After much eager research, the present inventors found that a protein having the ability to regenerate luciferin by acting on oxyluciferin and D-cysteine is present in living Coleoptera, and they successfully isolated and purified the protein.

Brief Summary Text - BSTX (14):

(1) A protein having the ability to regenerate luciferin by acting on oxyluciferin and D-cysteine.

Brief Summary Text - BSTX (15):

(2) A protein having the ability to regenerate luciferin by acting on oxyluciferin and D-cysteine, which is obtained by purifying an extract from a living Coleoptera of luminescence through purification steps including a chromatography.

Detailed Description Text - DETX (10):

The object of the present invention is as follows: a protein having the ability to regenerate luciferin by acting on oxyluciferin and D-cysteine is present in addition to the present invention, and by adding this protein to a luciferase reaction system the luminescence can persist and the amount of luciferin used can be reduced.

L10 2 L MIFERIN(5A) REGENERAT?

FILE 'WPIDS'

282 MIFERIN
84801 REGENERAT?

L11 2 L MIFERIN(5A) REGENERAT?

TOTAL FOR ALL FILES

L12 41 L MIFERIN(5A) REGENERAT?

=> s l12 not 2001-2003 PY

FILE 'MELLINE'

1241979 2 01-2003 PY

L13 2 L MELLINE NOT 2001-2003/PY

FILE 'SC1SEARCH'

2454464 2 01-2003 PY

L14 2 L SC1SEARCH NOT 2001-2003/PY

FILE 'LIFESCI'

232279 2 01-2003 PY

L15 2 L LIFESCI NOT 2001-2003/PY

FILE 'BIOTECHIDS'

56486 2 01-2003 PY

L16 2 L BIOTECHIDS NOT 2001-2003/PY

FILE 'BIOSIS'

1289472 2 01-2003 PY

L17 2 L BIOSIS NOT 2001-2003/PY

FILE 'EMBASE'

1118376 2 01-2003 PY

L18 2 L EMBASE NOT 2001-2003/PY

FILE 'HAILIUS'

2502531 2 01-2003 PY

L19 2 L HAILIUS NOT 2001-2003/PY

FILE 'NTIS'

26116 2 01-2003 PY

L20 2 L NTIS NOT 2001-2003/PY

FILE 'EPILOGUE'

721316 2 01-2003 PY

L21 2 L EPILOGUE NOT 2001-2003/PY

FILE 'BIOTECHNO'

15754 2 01-2003 PY

L22 2 L BIOTECHNO NOT 2001-2003/PY

FILE 'WPIDS'

2457159 2 01-2003 PY

L23 2 L WPIDS NOT 2001-2003/PY

TOTAL FOR ALL FILES

L24 12 L 2 NOT 2001-2003/PY

=> dup r 124

PROCESSING COMPLETED FOR L24

L25 6 DUP REM L24 (6 DUPLICATES REMOVED)

=> d tc

L25 ANTE R 1 OF 6 HCAPLUS COPYRIGHT 2003 ACS on STN
 TI Microorganism measuring method.
 SO Jpn. Kokai Tokkyo Koho, 11 pp.
 CEN/EN: JPKXN-1F
 IN Sakakibara, Tatsuya; Murakami, Shigeharu
 AN 1995-168956 HCAPLUS
 DN 1301234323
 PATENT NO. FIND DATE APPLICATION NO. DATE
 --- --- --- ---
 PI JP 11069994 AZ 19990316 JP 1997-316621 19971104

L25 ANTE R 2 OF 6 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN DUPLICATE 1
 TI *In vitro* release of a reporter gene for transformation studies in rice (*Oryza sativa* L.)
 SO PLANT CELL REPORTS, (MAY 1999) Vol. 18, No. 9, pp. 715-720.
 Publisher: SPRINGER VERLAG, 175 FIFTH AVE, NEW YORK, NY 10010.
 ISSN: 0721-7714.
 AU Baran-Wolff J; Harwood W A; Lonsdale D A; Harvey A; Hull R; Snape J W
 (Reprint)
 AN 1995-121621 SCISEARCH

L25 ANTE R 3 OF 6 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
 TI *Enzyme involved in regenerating firefly luciferin.*
 SO Official Gazette of the United States Patent and Trademark Office Patents, (S. pt. 2B, 1998) Vol. 1214, No. 5, pp. 5300.
 ISSN: 1068-1133.
 AU Kondo, M.
 AN 2002-066085 BIOSIS

L25 ANTE R 4 OF 6 HCAPLUS COPYRIGHT 2003 ACS on STN DUPLICATE 3
 TI Firefly protein involved in **regenerating luciferin**
 f: luciferin and D-cysteine
 SO Eng. Appl., 4 pp.
 CEN/EN: JPKXN-1F
 IN Kondo, M.; Kaki
 AN 1997-306406 HCAPLUS
 DN 1997-11083
 PATENT NO. FIND DATE APPLICATION NO. DATE
 --- --- --- ---
 PI EP 0212177 AZ 19980225 EP 1997-306406 19970821
 EP 0212177 A2 19990908
 AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT,
 IE, FI

L25 ANTE R 5 OF 6 HCAPLUS COPYRIGHT 2003 ACS on STN
 TI Preparation of protein associated with **regeneration** of
 luciferin from oxyluciferin and cysteine
 SO Jpn. Kokai Tokkyo Koho, 4 pp.
 CEN/EN: JPKXN-1F
 IN Kondo, M.; Kaki
 AN 1997-219375 HCAPLUS
 DN 1997-0814
 PATENT NO. FIND DATE APPLICATION NO. DATE
 --- --- --- ---
 PI JP 110791 AZ 19980506 JP 1997-219375 19970814

L25 ANTE R 6 OF 6 HCAPLUS COPYRIGHT 2003 ACS on STN
 TI Luciferase assay. Principles and practice
 SO Methods of Technical Analysis (1968), 16, 99-181
 CEN/EN: JPKXN-1A; ISSN: 0076-6941
 AU Sorenson, Richard L.
 AN 1997-219374 HCAPLUS
 DN 6 pp. 99-181

=> d a

L25 A 10 10 0 6 HCAPLUS COPYRIGHT 2003 ACS on STN
AB A simple, sensitive and rapid method is described for measuring microorganism trapped on filter membrane. Microorganism is trapped on filter membrane by filtering a sample liq. contg. microorganism through membrane. Biol. constituents are extd. from the trapped microorganism and placed on membrane. Then, luminescence generated on the membrane is measured after adding ATP generating reaction reagents and bioluminescence reagents. In this method, increased luminescence is obsd. by converting various adenosine-phosphate esters to ATP and by regenerating consumed ATP.

L25 A 10 10 0 6 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN DUPLICATE 1
AB Transfected rice plants of var 'TN1' were regenerated from immature embryos following particle bombardment with a construct containing the firefly luciferase gene as a reporter gene and the hygromycin resistance gene as a selectable marker. Expression of the luciferase gene in the leaves of the substrate luciferin was visualised in the calli derived from bombardied immature embryos and in the leaves and roots of the regenerated transformed plants using a low light imaging system (LLIS). Embryogenic callus proliferation and plant regeneration were unaffected by **luciferin** treatment and the rapid screening. The quantitative Luc assay using samples of leaf tissue from the segregating generations gave early information about the homozygous and hemizygous state of the luc transgene.

L25 A 10 10 0 6 BIOFIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
AB

L25 A 10 10 0 6 HCAPLUS COPYRIGHT 2003 ACS on STN DUPLICATE 3
AB Enzyme having the ability to **regenerate luciferin** from adenyloxyluciferin and D-cysteine was purified from 2 firefly species (L. luciferina and L. lateralis). The L. luciferina enzyme has pH and temp. of pH 7-8 and 35-50.degree., and retains .gtoreq.80% activity after thermal treatment at 50.degree. for 30 min, whereas L. lateralis enzyme has pH and temp. optima of pH 8-9 and 35-40.degree., resp., and retains .gtoreq.80% activity at 50.degree. for 30 min. Providing this protein to a luciferin/luciferase reaction system, luminescence can persist and the amt. of luciferase and luciferin is greatly reduced.

L25 A 10 10 0 6 HCAPLUS COPYRIGHT 2003 ACS on STN
AB Protein capable of **regenerating luciferin** from adenyloxyluciferin and D-cysteine is purified from fire fly lantern ext. (Sigma) by a series of chromatog. The protein exhibits a pH optimum of 8, temp. optimum 35.apprx.50.degree., and mol. wt. 40,000 by SDS-PAGE. It remains >80% active after incubating at 50.degree. for 30 min. The protein may improves the efficiency and duration of the reaction.

L25 A 10 10 0 6 HCAPLUS COPYRIGHT 2003 ACS on STN
AB In the firefly Photinus pyralis, the pyrophosphatase (I) hydrolyzed pyrophosphate (II) (endogenous or exogenous) with the release of light. With excess amts. of II, the light was weak, but the intensity increased as II was hydrolyzed. II also promoted the formation of adenyloxyluciferin (III) by luciferase with the utilization of ATP and oxidized **luciferin**. The addn. of luciferin and luciferin-AMP caused a flash of luminescence by the synthesis of ATP from III and ATP utilization in adeny-luciferin formation. Other reactions involving I are described. Procedures for ADP and ATP assay are considered. Sources of error in measurements of

File usage and procedures for controlling them are described.

| => fil | C | SINCE FILE | TOTAL |
|--|---|------------|---------|
| COST | D | ENTRY | SESSION |
| FULL FILE | D | 31.29 | 31.50 |
| DISCOUNTS AND FEES (FOR QUALIFYING ACCOUNTS) | | SINCE FILE | TOTAL |
| CA SUBJECTS | D | ENTRY | SESSION |
| | | -2.60 | -2.60 |

FILES ARE SHOWN. HEADLINES, WPIDS' ENTERED AT 14:52:37 ON 06 AUG 2003
ALL CONDITIONS ARE MET. NO RESTRICTIONS APPLY. SEE HELP USAGETERMS FOR DETAILS.

3 FILES IN THE FILE LIST

=> s 1
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FILE 1:
1: D AND PRY
2: D AND PRY<=2000
3: (PRY<=1,000)

L26 4: (D AND W) PC AND PRY<=2000

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3: (D AND W) PC AND PRY<=2000

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TOTAL FILES
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PROCESSED FOR L29
L30 4: (D AND W) PC AND PRY<=2000 (4 DUPLICATES REMOVED)

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L30 1: 2003-06-06 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT/ISI on STN
TI 2: cruciferae-originated genes encoding proteins capable of
3: emitting luciferin especially from oxyluciferin,
4: for producing recombinant DNAs and transformants to give proteins
5: in assaying adenosine triphosphate;
6: for mediated gene transfer and expression in host cell and DNA
7: for use in medicine and food hygiene

AU 1: HUPOSAWA K; KAIJIYAMA N
AN 2: 2003-06-06 BIOTECHDS
PI 3: 2003-06-06 Feb 2002

L30 1: 2003-06-06 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT/ISI on STN
TI 2: cruciferae-originated genes encoding proteins capable of
3: emitting luciferin especially from oxyluciferin,
4: for producing recombinant DNAs and transformants;
5: for use in medicine and food hygiene

AU 1: HUPOSAWA K; KAIJIYAMA N
AN 2: 2003-06-06 BIOTECHDS
PI 3: 2003-06-06 Feb 2002

L30 A1 WO 03041701 HCAPLUS COPYRIGHT 2003 ACS on STN
TI L-
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SO C-
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AN 20030417 HCAPLUS
DN 1 02
P1 J 02 34671 A2 20020205 JP 2000-228227 20000728
W 01 17481 A1 20020207 WO 2001-JP6455 20010726 <--
US
AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL,
PT, SE, TR
I 02 34674 A1 20030502 EP 2001-954353 20010726 <--
AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT,
IE, FI, CY, TR

L30 A1 WO 03041701 HCAPLUS COPYRIGHT 2003 ACS on STN
TI L-
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IN H-
AN 20030417 HCAPLUS
DN 1 02 17
P1 J 02 34671 A2 20020205 JP 2000-228226 20000728
W 01 17481 A1 20020207 WO 2001-JP6454 20010726 <--
US
AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL,
PT, SE, TR
I 02 34674 A1 20030502 EP 2001-954352 20010726 <--
AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT,
IE, FI, CY, TR

L30 A1 WO 03041701 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT/ISI on STN
TI L-
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AU C-
AN 20030417 BIOTECHDS
PI 01 17481 12 Apr 2001

L30 A1 WO 03041701 HCAPLUS COPYRIGHT 2003 ACS on STN
TI L-
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SO C-
IN C-
AN 20030417 HCAPLUS
DN 1 02
P1 J 01 17481 A1 20010726 WO 2001-JP238 20010117 <--
CN, DE
W: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL,
PT, SE, TR
C 01 17481 A2 20010807 JP 2000-28976 20000207
C 01 17481 A2 20011023 JP 2000-112790 20000414

| | | | | | |
|---|---|----|----------|----------------|--------------|
| S | 0116919 | A2 | 20011030 | JP 2000-119798 | 20000420 |
| S | 0116910 | A2 | 20011002 | JP 2000-362340 | 20001129 <-- |
| I | 74 04 | A1 | 20021211 | EP 2001-901364 | 20010117 <-- |
| | AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, CY, TR | | | | |
| U | 01164194 | A1 | 20030403 | US 2002-188091 | 20020703 <-- |

=> d ä

L30 AB A. AdK (U.S. HCAPLUS) COPYRIGHT 2003 ACS on STN
AB A. A regeneration reaction system wherein AMP is converted into ADP by
treatment with adenylate kinase (AdK) or polyphosphate:AMP
kinase (PPT) in the presence of a trace amt. of ATP and the
concomitant ADP is converted into ATP and a polyphosphate (polyP) compd. by
treatment with polyphosphate synthase in the presence of a polyphosphate
carrier. This disclosure is disclosed. Application of the reaction system in detection of
a nucleic acid nucleotide or RNA by using bioluminescence kit contg. firefly
luciferase and luciferin is described. RNA is degraded to mononucleotides
by DNase treatment prior to the use of the reaction system. The system
is considered an alternative to existing enzymic ATP regeneration systems in
which both malpyruvate and acetylphosphate serve as phosphoryl donors
and the advantage that AMP and polyP are stable, inexpensive
and readily available.

=> loc

| COST | IN S. DOLLARS | SINCE FILE ENTRY | TOTAL SESSION |
|---|---------------|------------------|---------------|
| FULL PAYMENT | 66.86 | 98.36 | |
| DISCOUNT AMOUNT (FOR QUALIFYING ACCOUNTS) | | SINCE FILE ENTRY | TOTAL SESSION |
| CA SURCHARGE | -0.65 | -3.25 | |